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Supplemental information

Distinct regulation of tonic GABAergic inhibition by NMDA receptor subtypes

Kunwei Wu, David Castellano, Qingjun Tian, and Wei Lu

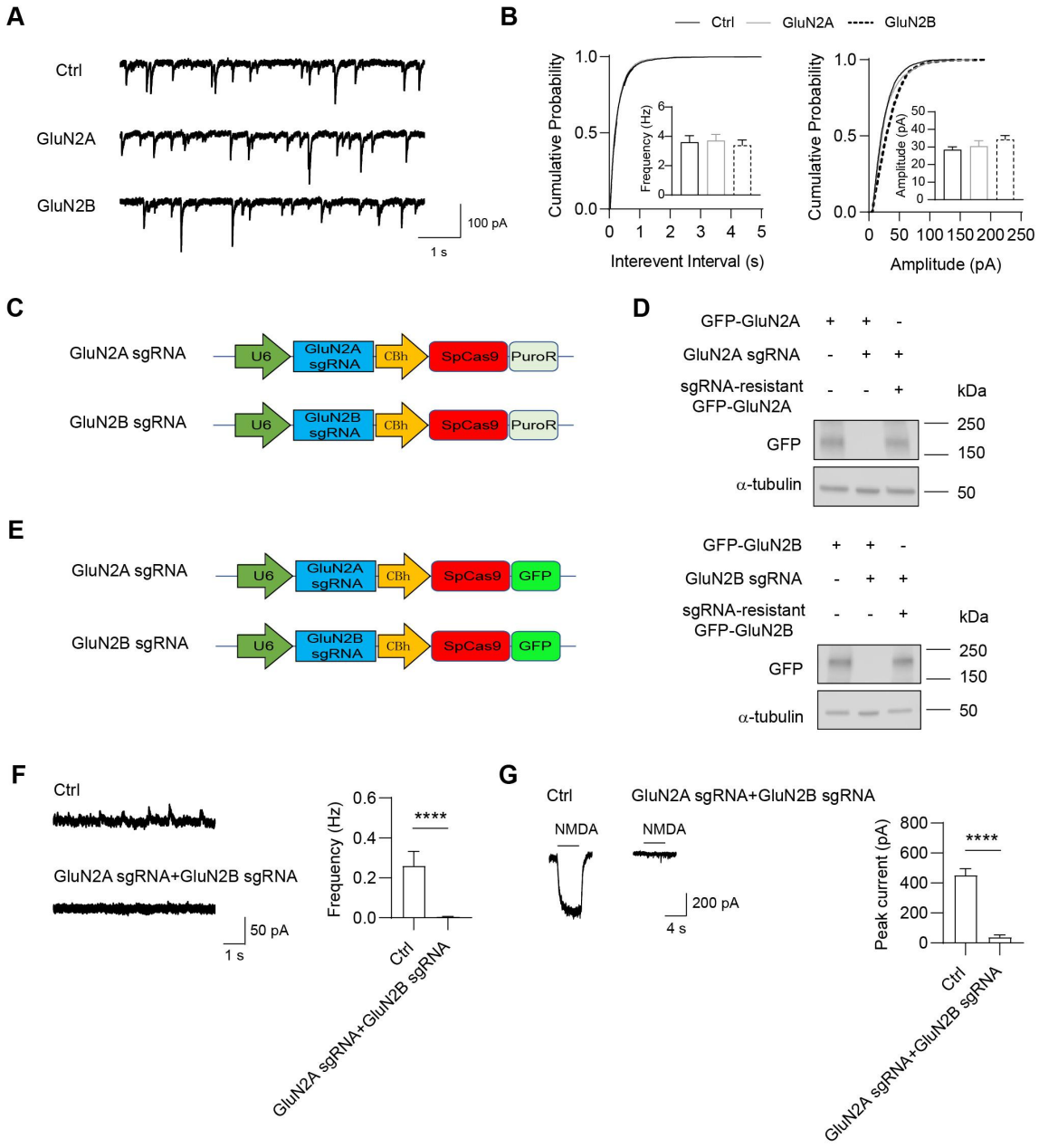

Figure S1. The effects of overexpression of GluN2A or GluN2B on phasic inhibition and validation of knockout efficacy of sgRNAs, Related to Figure 1

(A) Representative mIPSCs traces recorded in cultured hippocampal neurons expressing pCAG-GFP alone or pCAG-GFP together with GluN2A or GluN2B.

(B) mIPSCs mean frequency and cumulative probability plots of mIPSC interevent intervals (left). mIPSCs mean amplitude and cumulative probability plots of mIPSC amplitude (right). (n = 11-14 for each group, one-way ANOVA test with Dunnett's multiple comparisons test).

(C) Schematic diagram of CRISPR/Cas9 vector (without GFP tag) targeting GluN2A or GluN2B gene used for biochemical experiments.

(D) Representative Western blots of HEK293 cell lysates showing expression of GluN2A and GluN2B following co-transfection of indicated plasmids.

(E) Schematic diagram of CRISPR/Cas9 vector (with GFP tag) targeting GluN2A or GluN2B gene used for electrophysiological experiments.

(F) NMDA mEPSCs recorded in cultured hippocampal neurons transfected with GluN2A gRNA and GluN2B sgRNA as shown in E. (n = 9-10 for each group, Mann-Whitney U test, $p < 0.0001$).

(G) NMDA-evoked currents recorded in cultured hippocampal neurons transfected with GluN2A sgRNA and GluN2B sgRNA as shown in E. (n = 9-16 for each group, Mann-Whitney U test, $p < 0.0001$).

****$p < 0.0001$. All data are presented as mean ± SEM.

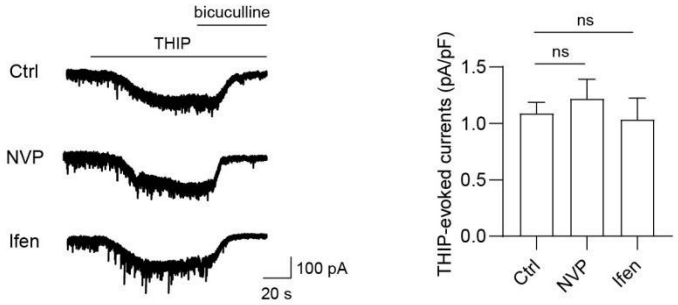

Figure S2. Pharmacological suppression of GluN2A- and GluN2B-NMDARs has no effect on δ-GABA$_A$R-mediated tonic inhibition, Related to Figure 2

Representative traces and summary graphs showing that NVP and Ifen treatment had no effect on THIP (3 μM, a GABA$_A$R agonist with a preference for δ-containing GABA$_A$Rs)-evoked currents. (n = 9-11 for each group, one-way ANOVA test with Dunnett's multiple comparisons test).

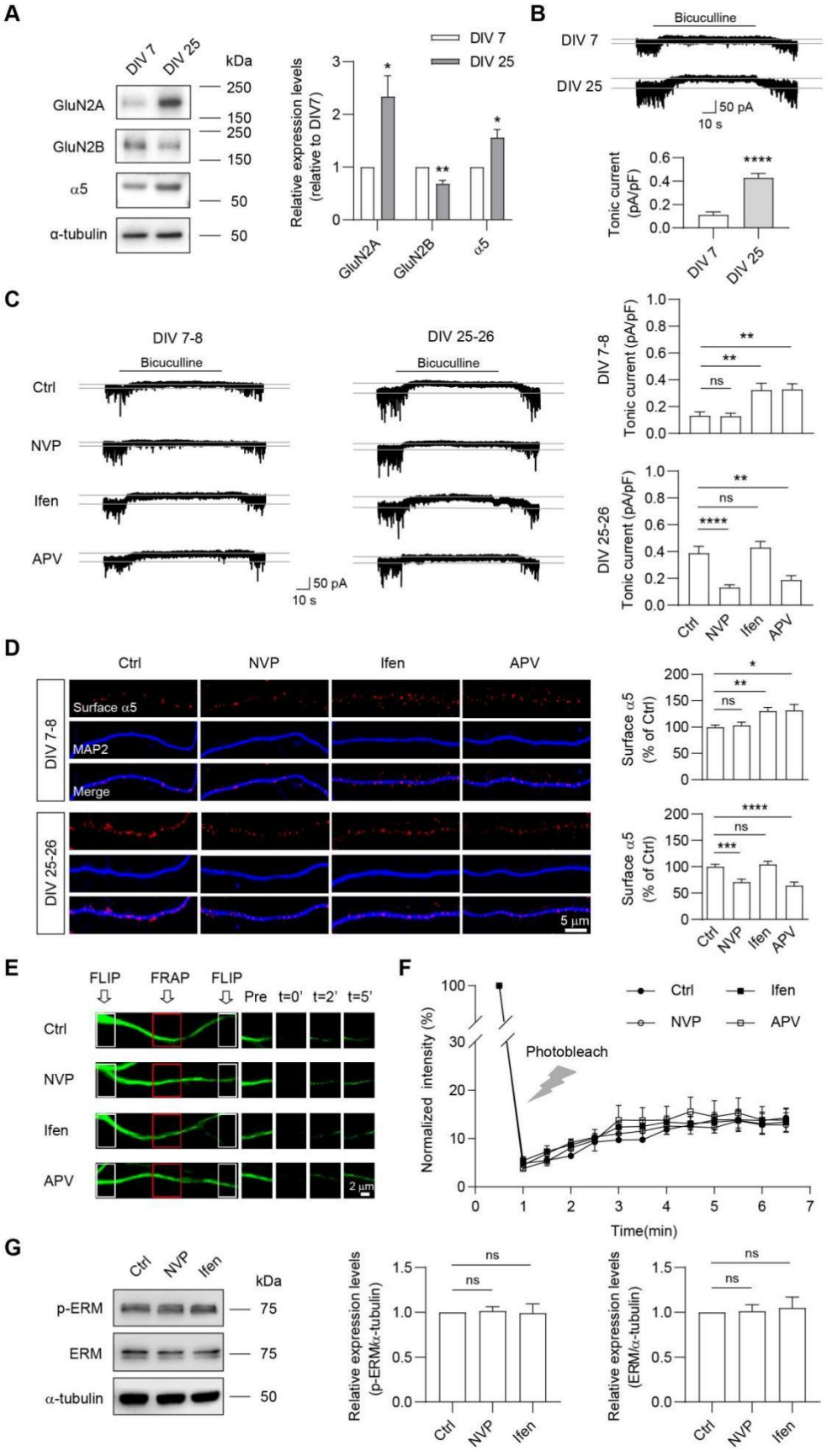
A
DIV 7
DIV 25
kDa
GluN2A
GluN2B
α5
α-tubulin
250
150
250
150
50
50
Relative expression levels (relative to DIV7)
DIV 7
DIV 25
GluN2A
GluN2B
α5
B
Bicuculline
DIV 7
DIV 25
50 pA
10 s
Tonic current (pA/pF)
C
DIV 7-8
DIV 25-26
Bicuculline
Ctrl
NVP
Ifen
APV
50 pA
10 s
Tonic current (pA/pF)
D
Ctrl
NVP
Ifen
APV
DIV 7-8
DIV 25-26
Surface α5
MAP2
Merge
5 μm
Surface α5 (% of Ctrl)
E
FLIP
FRAP
FLIP
Pre
t=0'
t=2'
t=5'
Ctrl
NVP
Ifen
APV
2 μm
F
Ctrl
NVP
Ifen
APV
Photobleach
Normalized intensity (%)
Time(min)
G
Ctrl
NVP
Ifen
kDa
p-ERM
ERM
α-tubulin
75
75
50
Relative expression levels (p-ERM/α-tubulin)
Relative expression levels (ERM/α-tubulin)
ns

Figure S3. Development-dependent regulation of tonic inhibition by GluN2A- and GluN2B-NMDARs, Related to Figure 2

(A) Representative Western blots and summary graphs showing the expression level of GluN2A, GluN2B and α5-GABA$_A$R in the homogenates of hippocampal neurons at DIV7 and DIV25. (n = 3 independent experiments, GluN2A: t test, p = 0.0273; GluN2B: t test, p = 0.0069; α5: t test, p = 0.0202)

(B) Representative traces and summary graphs showing tonic currents in cultured hippocampal neurons at DIV7 and DIV25. (n = 8-10 for each group, Mann-Whitney U test, p < 0.0001)

(C) Representative traces and summary graphs showing the changes of tonic currents in immature (DIV7-8, n = 10 for each group, one-way ANOVA test, $F_{(3, 36)}$ = 9.211, p = 0.0001 with Dunnett's multiple comparisons test, Ctrl versus Ifen, p = 0.0025; Ctrl versus APV, p = 0.0018) or more differentiated (DIV25-26, n = 10-12 for each group, one-way ANOVA test, $F_{(3, 40)}$ = 16.00, p<0.0001 with Dunnett's multiple comparisons test, Ctrl versus NVP, p < 0.0001; Ctrl versus APV, p = 0.0017) hippocampal neurons under treatments of NVP, Ifen or APV.

(D) Immunostaining and summary graphs showing changes of surface α5 expression in immature (DIV7-8, n = 32-39 for each group, Kruskal-Wallis test with Dunnett's multiple comparisons test, Ctrl versus Ifen, p = 0.0014; Ctrl versus APV, p = 0.0197) or more differentiated (DIV25-26, n = 22-24 for each group, Kruskal-Wallis test with Dunnett's multiple comparisons test, Ctrl versus NVP, p = 0.001; Ctrl versus APV, p < 0.0001) hippocampal neurons under treatments of NVP, Ifen or APV.

(E) Representative images (left) of SEP-α5 fluorescence and the regions of the neuronal dendrites used for the fluorescence recovery after photobleaching (FRAP) and fluorescence loss in photobleaching (FLIP) experiments. Repetitive photobleaching (FLIP, white box) occurred at regions bilateral to the central FRAP region (red box). Each column (right) represents before (pre), immediately after (t = 0'), and at 2 min (t =2') and 5 min (t = 5') after photobleaching in each condition.

(F) Normalized fluorescence recovery curves showing that NVP, Ifen and APV had no effects on α5 exocytosis. (n = 6 for each group, two-way ANOVA test with Dunnett's multiple comparisons test)

(G) Representative Western blots and summary graphs showing that NVP and Ifen treatment had no effect on ERM and p-ERM expression. (n = 3 independent experiments, one-way ANOVA test with Dunnett's multiple comparisons test).

*p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001. All data are presented as mean ± SEM.

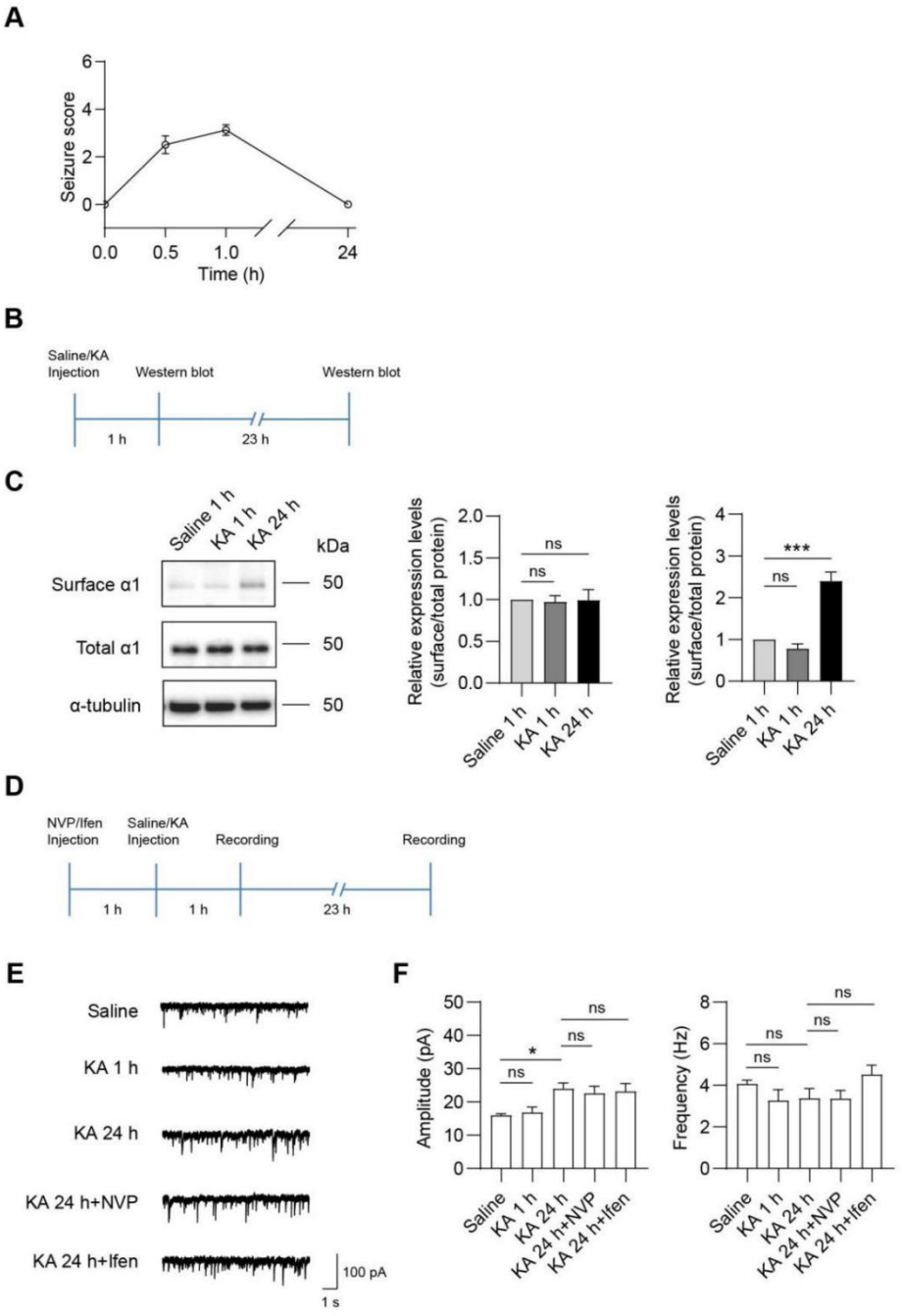

Figure S4. Pharmacological suppression of GluN2A- and GluN2B-NMDARs has no effect on phasic inhibition in hippocampal CA3 neurons in a KA-induced seizure model, Related to Figure 4

(A) Seizure score was evaluated at 0 h, 0.5 h, 1 h and 24 h after KA injection according to the modified Racine scale.(n = 8)

(B) Experimental design for Western blot.

(C) Representative Western blots and summary graphs from cell-surface biotinylation assays showing surface and total α1-GABA$_A$R expression in KA-induced seizure model. (n = 3 independent experiments, one-way ANOVA test, $F_{(2, 6)} = 39.89$, $p = 0.0003$ with Dunnett's multiple comparisons test, Saline 1 h versus KA 24 h, $p = 0.0007$)

(D) Experimental design for electrophysiological recording.

(E) Representative mIPSCs traces recorded in hippocampal CA3 neurons in acute brain slices.

(F) Summary graphs showing mean amplitude and frequency of mIPSCs. (n = 10-16 for each group, one-way ANOVA test, $F_{(4, 55)} = 4.576$, $p = 0.0029$ with Tukey's multiple comparisons test, Saline versus KA 24 h, $p = 0.0203$).

*$p < 0.05$ and ***$p < 0.001$. All data are presented as mean ± SEM.